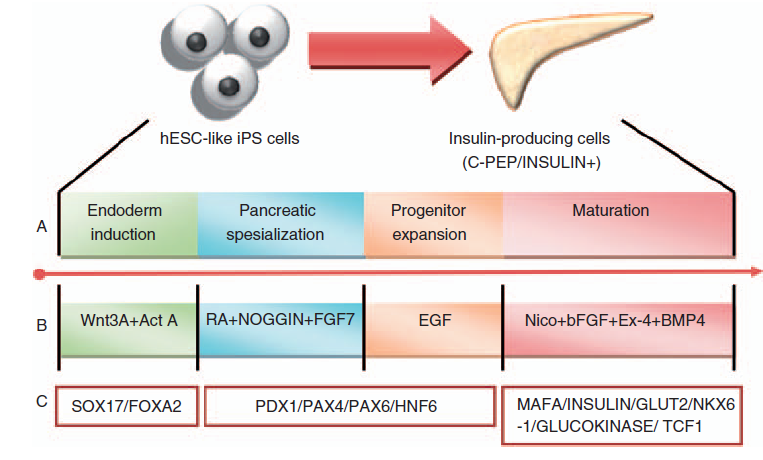
**Treatment of Diabetes by β-cell Transplantation from iPS Cells**

1. How do induced pluripotent (iPS) cells differ from embryonic stem (ES) cells?
   1. iPS cells & ESCs
      1. Similarities
         1. Morphology
         2. Proliferation
         3. Expression of similar genes to ESC cells
         4. Can differentiate into cells of all three germ layers
      2. Differences
         1. Different overall gene expression
         2. Could not product adult chimeras
         3. Originally taken from somatic tissue & transformed
            1. The undifferentiated (use the Delorean!)
2. Compare the advantages and disadvantages of using iPS vs ESC for therapeutic transplantation.
   1. iPS Advantages
      1. Use cells directly from patient
         1. Overcome immune rejection in transplantation
      2. Allow the discontinued use of ESCs
      3. Can use to culture cell types and test new drugs
      4. Generation of disease models
         1. Can help elucidate disease mechanism
      5. Simplicity 🡪 produced in lab & can produce many clones of the cell line
   2. ESC Advantages
      1. Directly from the patient
      2. Are completely pluripotential
         1. Can differentiate into any type of cell
   3. iPS Disadvantages
      1. Teratoma formation
         1. Germ cell tumor with many cell types (from undifferentiated cells)
      2. Activation of oncogenes possibly
      3. iPS cells derived from certain somatic cells may be better at differentiating into different cell types
   4. ESC Disadvantages
      1. Teratoma formation
      2. Controversy about destroying/ using potential human life
3. Describe the different methods of creating iPS, their advantages and disadvantages.
   1. Retroviruses/Lentiviruses
      1. Most iPS cells = somatic cells transformed with retroviruses carrying transgenes
         1. Transgenes = incorporate into DNA
         2. Oct3/4 = most important transcription factor for iPS cell induction
   2. Adenoviruses
      1. Efficiency is EXTREMELY low in producing iPS cells
   3. Plasmids
      1. No integration of plasmid in host DNA
         1. Can be forced to integrate using certain conditions
      2. Efficiency is EXTREMELY low in producing iPS cells
   4. Chemicals to induce factor expression
      1. Only a few & not as effective
   5. Need to analyze genome in all these to check for mutations and other abnormalities
   6. Cells used
      1. Mouse = Fibroblasts, bone marrow cells, hepatocytes, gastric epithelial cells, pancreatic cells, neural stem cells, & B-lymphocytes
      2. Human = Blood progenitor cells, skin fibroblasts, & keratinocytes
4. What are some of the hazards of using iPS?
   1. Formation of teratoma
   2. Incomplete reprogramming of somatic cells to iPS could result in impaired differentiation
   3. Retroviruses/Lentiviruses
      1. Activation of transgenes could lead to tumorigenesis
      2. Viral gene integration could cause gene activation
   4. Safely obtain cells (blood draw/ biopsy)
   5. Differentiation/ induction capacity of different cell types after obtaining
   6. Cell types with different integration sites for introduced gene (some = few sites)
5. How can these hazards be minimized?
   1. Hematopoietic Stem cells & mesenchymal stem cells = do not form teratoma
      1. If we can generate other cell types from these = don’t have to use iPS cells
      2. Would stop teratoma formation
   2. Use of other methods (plasmids & adenovirus vectors) to prevent insertion of DNA into important genes
   3. Transforming one adult somatic cell into another = transdifferentiation
      1. Ultimate goal = eliminate need for iPS cells & stop teratoma formation
6. Describe the sources of cells used to generate iPS cells for treatment of diabetes.
   1. Dermal fibroblasts
   2. Keratinocytes
   3. Skin fibroblasts
7. Describe how iPS cells are induced to differentiate into insulin-producing β cells.
   1. Acquire somatic cells
   2. Transfect cells with 4 transcription factors (Oct4, Sox2, Lin28, & Nanog
      1. Turn on pluripotency genes
   3. Pluripotent cells stimulated to differentiate in β-cells
      1. EGF, bFGF, Exendin-4, nicotinamide, & BMP4



1. Explain why rejection of even autologous β cell transplant is problematic in T1DM.
   1. The immune system of the receiving patient will attack the newly formed β-cells that have been transplanted into the patient thus resulting in their destruction
      1. This makes transplant of cadaveric or even regenerated pancreatic β-cells by iPS hard to sustain in the long term
2. Describe solutions to immune rejection of transplanted beta cells for treatment of T1DM.
   1. Immunosuppressive drugs
   2. Cell-base microencapsulation technology
      1. Package transplanted tissue or cells into a semipermeable membrane coating
         1. Provides barrier from attack
         2. Yet allows for diffusion of substances
   3. Induction of immune tolerance by antigen introduction
      1. Introduces β cell antigen into the thymus to produce self tolerance
         1. Self tolerance = body will not fight/reject organ